Organogenesis and Growth Response of Brassica *oleracea* var. *Italica* through *in Vitro* Culture

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***Abstract*—**Brassica oleracea var.Italica or commonly known as broccoli is an edible plant with very commercial value. Plant tissue culture is the best alternative to propagate broccoli in low land area. This research aims to study organogenesis of broccoli through in vitro culture. The seeds were serially sterilized using 70%, 50%, 20% and 10% sodium hypochlorite. Subsequently, the seeds were sterilized using 70% alcohol. Sterilized broccoli seeds were cultured onto Murashige and Skoog, 1962 (MS) basal medium. In vitro response of explant growth was observed. Seed germination was observed within day 12 of seed culture. Elongation of shoots was observed after three to four week of culture. Complete plantlets were observed after 6 weeks of culture. Further organogenesis response was observed when shoot, stem and root explants of eight-week-old aseptic seedlings were then transferred onto Murashige and Skoog medium added with combination of Naphthalene Acetic Acid (NAA) and Benzylaminopurine (BAP). Micropopagation of Brassica oleracea var. Italica was successfully achieved through plant tissue culture process and proven to be a good alternative to conventional planting.

***Keywords*—**organogenesis, in vitro, Murashige and Skoog medium, seedlings, plantlets

I. Introduction

BRASSICA oleracea var italiaca or commonly known as Broccoli is derived from Brassica family. Broccoli has many benefits which it is very nutritious to consumer [1]. Broccoli has high content of antioxidant such as sulforus, beta-carotene, and indole. By taking food that contain antioxidant regularly, the percentage of oesophagus and stomach cancer can be reduced. Besides, antioxidant helps to prevent the harmful effects from the by-product of oxidation [2]. Carotenes, vitamin C and E are the food that contains major antioxidant that are beneficial to human health. This showed that broccoli has high important value in daily intake.

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Broccoli are highly vulnerable to high temperature and thse vegetable crops grow best in cool climate [3]. In order to propagate broccoli in low land area, plant tissue culture or organogenesis technique can be used as a platform. Plant tissue culture also referred to as in vitro, axenic or sterile culture [4]. Organogenesis has been widely reported as the most popular method for the formation of Brassica crops. Organogenesis is a technique to produce a plant using explants in sterile or aseptic media and environment. According to [5], organogenesis can be used to overcome lack of seedling problems. By organogenesis, plants that are free from diseases can be produced. Besides, organogenesis also can enhance the growth of plant that is difficult to growth in normal climate.

II. Material And Method

Preparation of MS (Murashige & Skoog, 1962) medium:

800ml of distilled water was filled up in duran bottle. Thirty grams of sucrose was added to 800ml of distilled water. Eight grams of agar was added into the solotion followed by 4.4 grams of Murashige and Skoog medium. Distilled water was added into the solution so that the amount of solution in 1L. The pH was adjusted to about 5.8 by adding hydrochloric acids to lower the pH value or sodium hydroxide to increase the pH value. The solution was stirred and heated for a while to ensure that the agar is completely melted. The medium was autoclaved for 20 minutes at 121^{O}C.

Seed preparation:

Explant needs to be surface sterilized before inoculated into the medium agar to prevent contamination. This process was done in the laminar flow. Before that, the seed need to sterelized outside the laminar flow first. Tap water, 70%, 50%, 20%, and 10% sodium hypochlorite, 70% ethanol, and distilled water were prepared before the experiment started. Seed were rinsed under running tab water for 60 minutes. After that seeds were surface sterilized with 70%, 50%, 20% and 10% sodium hypochlorite for 5 minutes followed by rinsing with distilled water subsequently. In the laminar flow, seeds were surface sterilized with 70% ethanol followed by sterile distilled water for 3 times. The seeds were then cultured on germination medium consist MS salts, [6] sucrose and agar.

After that, sees were transferred into jars that contain the media. The jam jar was tightly capped using paraflim to avoid any contamination. Germination of seeds will be observed. All

cultures will be incubated in the culture room at 20°C ± 1°C of 16 hours light and 8 hours dark photoperiod.

TABLE I
MEAN PERCENTAGE OF CALLUS, NUMBERS OF SHOOTS AND NUMBER OF ROOTS FROM LEAVES, STEM AND ROOT EXPLANTS

Hormone concentration (mg/L) NAA + BAP	explant	% callus	No of shoot	No of root
0.5mg/L + 0.5mg/L	Leaves	27.15±7.76	-	6.23±0.68
	Stem	97.62±1.96	-	6.15±0.87
	Root	96.67±3.33	-	7.80±0.79
0.5mg/L + 1.0mg/L	Leaves	28.60±7.21	2.00±0.58	6.11±0.77
	Stem	86.20±4.34	1.57±0.43	7.90±0.77
	Root	89.47±7.23	-	5.00±0.58
0.5mg/L + 1.5mg/L	Leaves	40.71±11.83	-	8.33±2.20
	Stem	90.00±10.00	3.00±0.00	1.00±0.00
	Root	87.63±12.38	-	3.17±0.70
0.5mg/L + 2.0mg/L	Leaves	72.50±7.60	-	4.63±0.41
	Stem	98.00±0.95	-	4.88±1.34
	Root	98.00±1.67	-	3.70±0.37
1.0mg/L + 0.5mg/L	Leaves	47.83±7.52	1.00±0	5.24±0.53
	Stem	99.50±0.37	-	4.08±0.59
	Root	100.00±0.00	-	3.46±0.38
1.0mg/L + 1.0mg/L	Leaves	56.79±7.73	-	3.73±0.44
	Stem	99.17±0.68	-	2.94±0.36
	Root	100±0	-	4.41±0.38
1.0mg/L + 1.5mg/L	Leaves	45.17±8.05	-	5.23±0.35
	Stem	71.50±7.60	3.75±0.61	5.50±0.38
	Root	100.00±0.00	-	7.42±0.61
1.0mg/L + 2.0mg/L	Leaves	12.47±1.87	-	4.45±0.47
	Stem	99.00±1.00	-	4.07±0.78
	Root	100.00±0.00	-	2.63±0.52
1.5mg/L + 0.5mg/L	Leaves	34.53±7.10	-	6.19±0.62
	Stem	90.36±3.83	3.50±0.96	4.00±0.43
	Root	100.00±0.00	-	2.76±0.25
CONTROL TREAEMEMT 0.0mg/L + 0.0mg/L	Leaves	-	-	-
	Stem	-	3.60±0.58	1.8±0.37
	Root	-	-	2.27±0.27

Explants preparation:

After 6 weeks of germination, explants will be excised for further culture. Leaves stem and root explants will be taken from aseptic seedling and transferred into MS medium supplemented with various concentration of NAA ad BAP.

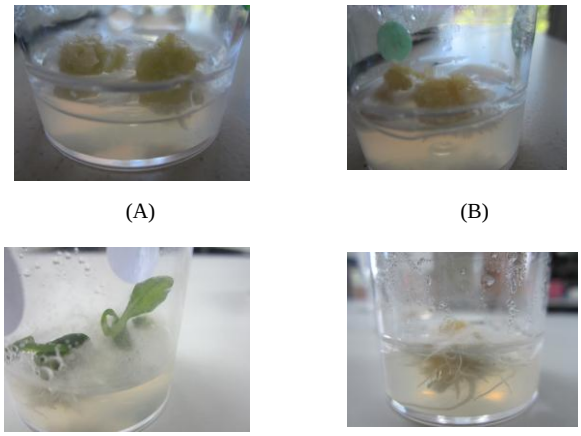
III. RESULTS AND DISCUSSION

Aseptic seedling of Brassica oleracea var. Italica was observed after 12 days. The plantlet were obtain after 6 weeks of culture. In previous studies there were different part of explants that have been used for regeneration, including peduncle explants [7], hypocotyls [1], shoot tip [8] and leaf tissue [9]. In this study the plant parts such as leaves, root and shoot were taken as source of explants. Hormone can be used as growth regulator to the explants. In this study, hormone Napthalene Acetic Acid (NAA) and Benzylaminopurine (BAP) were used as plant growth regulator. Explants were placed on MS medium added with various concentrations of NAA and BAP to study the percentage of callus induction, number of shoot and number of root of the explants. The success in callus induction is affected by type of plant material used and the in vitro condition [10]. The lowest concentration of 0.50mg/L NAA + 0.50mg/l BAP and the highest concentration was 1.5mg/L NAA +0.5mg/L BAP. After four week the explants were transferred, the data was recorded. The total numbers of explants used were 30 for each part of explants.

Among the combinations of NAA and BAP hormone, 1.0mg/L NAA+0.5mg/L BAP, 1.0mg/L NAA +1.5mg/L BAP, 1.0mg/L NAA +2.0mg/L BAP and 1.5mg/L NAA + 0.5mg/L BAP showed highest percentage of callus formation which are 100.00±0.00% where all the explants induced callus when root was used as source of explants Fig. (A) and Fig. (B). Meanwhile, control treatment (no hormone added) showed the result of 0.00±0.00 % of callus.

For number of shoot, there are only a few numbers of explants that were responsive to the combination of NAA+BAP concentration. Stem explants was the most responsive explants when cultured on MS medium containing 1.0mg/L NAA + 1.5mg/L BAP with 3.75±0.61 (C).

As shown in the data, the best concentration for root formation was 0.5mg/L NAA +1.5mg/L BAP which produced 8.33±2.20 of root (D). While control treatment gave the lowest number (0.00±0.00)



(A) (B)

(C) (D)

Fig. Formation of (A) and (B) callus from stem, (C) shoot formation from stem and (D) root formation

IV. CONCLUSION

In this study, micropropagation and callus induction of Brassica oleracea var. Italica was successfully achived. The right combination on NAA and BAP used will help the growth and development of *Brassica oleracea* var. *Italica in vitro*

ACKNOWLEDGMENT

The authors would like to thanks the Department of Agriculture Science, Faculty of Technical and Vocational Education, Sultan Idris Education University (UPSI), Malaysia for providing the research facilities.

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